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Taste Perception: How to Make a Gourmet Mouse

Dispatch

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Sugars and amino acids are mainly associated with desirable taste sensation. A new study using knockout mouse models shows that the detection of various sugars, artificial sweeteners and L-amino acids is exclusively mediated by taste cells that express one or pair-wise combinations of three G protein coupled receptors, T1R1, T1R2 and T1R3.

Humans can taste and discriminate among five distinct taste modalities: sweet, sour, salty, bitter, and umami, the taste of a few amino acids such as monosodium glutamate (MSG) and aspartame [1,2]. Recent functional studies have established that sweet taste is mainly, and perhaps exclusively, mediated by a small family of three G protein coupled receptors, the T1Rs. They are found exclusively in subsets of taste cells arranged in taste buds present primarily in various areas of the tongue [3]. *In situ* hybridization analysis has shown that the three T1R genes are expressed in three distinct combinations: cells expressing T1R1 and T1R3; cells expressing T1R2 and T1R3; and cells expressing only T1R3 [4,5]. Moreover, calcium imaging of HEK 293 cells expressing T1R2 and T1R3 has shown that these two proteins form a functional receptor for various sugars and artificial sweeteners [4,6]. Finally, the mouse homolog of T1R3 is the *sac* gene, known to be a major genetic determinant of responses to artificial sweeteners and sugars [4,5,7–9].

Whereas the role for the T1Rs in sweet perception has been widely accepted, different views have been expressed about the detection of umami taste. The first proposes that this savory taste, which is elicited mainly by the amino acid glutamate, is mediated by the pair-wise combination of T1R1 and T1R3 [6,10]. A second view is that the major component for detecting umami taste is a truncated form of the metabotropic glutamate receptor 4 (mGluR4) gene [11]. Confounding the complexity of the specific roles of these G protein coupled receptors in sweet and umami sensation in humans or attractive behavioral responses in rodents is the finding that sequence variations of these receptors have significant impact on taste perception. For example, mouse T1R1 and T1R3 respond to most L-amino acids, yet not all taste the same to humans: some are perceived as sweet (attractive to mice), some as neutral and yet others are even perceived as bitter (averse to mice), and only a few elicit umami taste (also attractive to mice).

The laboratories of Charles Zuker and Nicholas Ryba [12] have now collaborated to elucidate the role of each of the three T1R genes. They have done this using behavioral paradigms, such as the lick rate; by making electrophysiological recordings from the chorda tympani, a major gustatory nerve; and using gene knockout mouse models. The T1R3 gene has also been knocked out by Margolskee and co-workers [13], with findings similar to those reported by the Zuker/Ryba team [12]. None of the three T1R knockout mice showed any abnormalities or developmental defects; moreover, the detection of bitter, salty and sour tastes was not affected in these mice.

Table 1. The function of T1Rs as taste receptors.

Receptor	Cells	Function	Refs
T1R1 only	No	–	[4,12]
T1R2 only	No	Low affinity sugar receptor	[4,12]
T1R3 only	Yes	Low affinity sugar receptor	[4,12]
T1R1 + T1R2	No	–	[4,12]
T1R1 + T1R3	Yes	Umami/ L-amino acid receptor	[4,6,10,12]
T1R2 + T1R3	Yes	High affinity sugar and artificial sweetener receptor	[4–6,12]

Umami Taste

The Ryba/Zuker team [12] found that, in T1R1 or T1R3 knockout mice, the nerve response elicited by MSG was found to be reduced but not diminished. Similarly, the preference to MSG in behavioral assays was reduced, though not completely abolished. Significantly, the characteristic enhancement of umami sensation by 5′-inosine monophosphate (IMP) was completely abolished in both T1R1 and T1R3 knockout mice, a result also obtained by the Margolskee group [13].

Nevertheless, residual MSG responses in both knockout mice seemed to leave open the possibility that other umami receptors exist, and the Ryba/Zuker team [12] addressed this issue in detail. In this context, it is relevant to point out that MSG also contains sodium salt and, hence, elicits also salty taste. The authors therefore applied the sodium channel blocker amiloride along with MSG, and indeed found little residual nerve response or behavioral attraction in the T1R3 knockout mice. Moreover, other umami substances lacking sodium salt — L-Asp, MPG or L-AP4 — were found to elicit no responses in knockout mice in electrophysiological recordings from the chorda tympani. As expected, such mice show no preference for these chemicals over water in behavioral tests. From these additional experiments, the authors concluded that umami taste of MSG and its agonists is mediated solely by T1R1 and T1R3 (T1R2 knockout mice showed no defect in umami perception, either behaviorally or electrophysiologically; see below).

Sweet Taste

In T1R2 or T1R3 knockout mice, nerve responses to various sweeteners were abolished, suggesting that

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Figure 1. The taste of sweet and umami. Umami sensation is a strong indicator for protein-rich food (left). In contrast, 'sweet' is the combined flavor of many carbohydrates typically found in desserts.

both receptors are required for detection of artificial sweeteners; as expected, the lick rate of T1R2 or T1R3 knockout mice was reduced to base levels (those elicited by water). In contrast, response to sugars was strongly reduced at low concentration, but only partially reduced at increasingly higher concentrations. T1R2/T1R3 double knockout mice, however, lost both behavioral and electrophysiological responses to all sugars tested. These data suggest that T1R2 and T1R3 form a functional receptor for artificial sweeteners and a high-affinity sugar receptor, whereas either receptor alone can function as a low affinity sugar receptor (Table 1), a suggestion the authors were able to confirm for T1R3 by heterologous expression in HEK-293 cells [12]. It is worth noting that a significant fraction of taste cells express only T1R3, suggesting that this receptor indeed functions as a relevant low-affinity sugar receptor *in vivo* (neither T1R2 nor T1R1 is expressed singly in any taste cell).

The Gourmet Mouse

An interesting variation in sweet taste perception is revealed by a few chemicals. For example, the proteins monellin and thaumatin, as well as aspartame and neohesperidin dihydrochalcone, all taste sweet to humans, but none of these chemicals is attractive to rodents. This variation in sweet taste sensation is likely caused by the considerable sequence divergence of the T1R genes between the two species, as they are about 30% dissimilar in sequence; T1R2 in particular is implicated, as it is the only one that is exclusively involved in sweet taste perception.

To directly address this hypothesis, the Zuker/ Ryba [12] team generated transgenic mice expressing the human T1R2 gene under the control of the mouse T1R2 promoter. T1R2 knockout mice expressing this hybrid gene are now attracted to monellin, thaumatin and aspartame, indicating that the human T1R2 receptor indeed mediates the sweet taste quality of these chemicals. These mice still, however, fail to sense neohesperidin dihydrochalcone, suggesting that the taste of at least some sweet substances is mediated by T1R3 — or possibly by T1R3-induced modification of T1R2 — a notion further supported by the observations that HEK-293 cells expressing the human T1R2/T1R3 receptor pair show a robust response to neohesperidin dihydrochalcone.

To take this gain-of-function analysis one step further, the authors [12] created mice expressing in T1R2-expressing (sweet-sensing) taste cells a modified κ-opioid receptor activated solely by the synthetic ligand RASSL [14]. In these animals, the RASSL agonist spiradoline induced behavioral attraction, similar to that observed with sugars, whereas wild-type mice fail to show any responses to this ligand. These data strongly suggest that the κ-opioid receptor-expressing animals sense spiradoline as 'sweet', supporting the idea that cell identity defines taste perception.

These elegant *in vivo* studies, together with earlier reports by the same laboratories [15], allow us to draw an increasingly clearer picture of taste perception in mammals. There is a simple logic, not unlike that of the chemosensory system in the nematode *Caenorhabditis elegans* [16], whereby different taste cells are determined — by virtue of which genes they express — to mediate sweet, umami or bitter sensation. Activation of each taste cell population simply elicits the sweet, umami or bitter sensation, respectively (Table 1). The next major issue that is likely to see increased attention from taste researchers is that of how the apparently clear separation of these three taste modalities in the sensory cells is conveyed to the gustatory cortex via the brain stem and the thalamus. For example, it is not yet known how many types of taste cell are contacted by individual afferent fibers. What has been established is the fact that a single gustatory fiber innervates multiple taste buds, and within each taste bud, multiple taste cells [1].

Given the results reported by Zuker/Ryba [12], it would seem most effective if individual afferent fibers were to contact a single cell type: cells expressing either a specific combination of T1R genes or the T2R genes (Table 1). Electrophysiological recordings, however, have shown that individual afferent fibers respond to multiple stimuli, albeit one stimulus elicits clearly higher responses than the others [17]. Visualization of individual afferents in combination with taste receptor expression analysis or transgenic expression of trans-neuronal tracers in specific taste cell types [18,19] — albeit perhaps technically challenging — should become possible and eventually provide tools that might reveal a first view at the circuitry in the taste centers in the brain.

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